

Research Animal Standard Operating Procedures (SOP) must meet the following criteria:

- 1. Describe procedures or activities involving research animal(s) common to more than one research project.
- 2. Support the handling and or performance or undertaking of a procedure(s), involving an animal, in the same way on each occasion it is performed.
- 3. Describe a procedure or activity involving a research animal(s) undertaken by more than one person; and
- 4. Describe a procedure or activity involving a research animal(s) that will be undertaken in more than one location.

Name of Procedure	Behavioural tests – Elevated Plus Maze	
Species	Rodents	
	Reference	SOP#35 – Mar 22 - Elevated Plus Maze- rodents
	Author	Eugene Nalivaiko
	Version	1.2
	Date approved	25 March 2022
ACEC	Date for review	24 March 2025
	Procedure classification 1. Observation involving minor interference 2. Animal unconscious without recovery 3. Minor conscious intervention 4. Minor surgery with recovery 5. Major surgery with recovery 6. Minor physiological challenge 7. Major physiological challenge	1
Ethical considerations	 Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. The procedure must be performed according to current best practice to support the wellbeing of the animal. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent. 	

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Purpose

The purpose of this behavioural tests is to measure the level of anxiety in rodent subjects by specifically monitoring exploration behaviour and locomotor activity during exposure to classical elevated plus maze behavioural tests:

Elevated Plus Maze

Used to measure exploration activity in the experimental subject based on conflicting tendencies to explore a novel enclosed environment and to avoid elevated open spaces that ethologically mimic a high-risk situation. The two enclosed arms of the apparatus promote safety and allow the subject to explore, however the two juxtaposed open arms of the apparatus are more stressful and inhibit exploration behaviour. Elevated plus maze tests can therefore measure changes in exploration activity with open arm avoidance and depressed exploration behaviour indicating high levels of anxiety. The elevated plus maze is also an important 'control' test for other behavioural tests that can be affected by general anxiety-like behaviour, such as the social interaction or novel object recognition tests.

Description of procedure

The following is a description of the Elevated Plus Maze behavioural test used on subject rodents. Note this is an observational experiment only and does not describe any prior/post-surgical interventions:

Elevated Plus Maze Equipment

- 1. Elevated Plus Maze apparatus attached to a steel frame stand
- 2. Video recorder mounted to the steel frame positioned above the maze
- 3. Computer for post recording analysis
- 4. Video tracking software (such as CAT track or Ethovision)
- 5. Timer

Elevated Plus Maze Apparatus- Rat

The "elevated plus maze" experimental apparatus is constructed from dark coloured plastic comprising a 5cm x 5cm central square with four 5cm wide x 45cm long arms radiating from the central square. Two of the arms in the same plane are enclosed by 15cm high dark coloured plastic walls (closed arms). The other two perpendicular arms remain open (open arms) but have a 2.5cm high edge placed around the open arms to prevent the subjects from falling from the maze. A white line to measure distance travelled is draw on each arm at 22.5cm being halfway from the central square of each radiating arm. The entire maze apparatus is elevated 45cm above the floor on a plus shaped steel frame stand. The video camera for recording movement is mounted to the steel frame suspended above the maze. A schematic representation of the elevated plus maze apparatus with dimensions and apparatus setup is depicted below (see Image 1. & 2).

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Elevated Plus Maze Apparatus- Mouse

The "elevated plus maze" experimental apparatus is constructed from dark coloured plastic comprising a 5cm x 5cm central square with four 5cm wide x 30cm long arms radiating from the central square. Two of the arms in the same plane are enclosed by 15cm high dark coloured plastic walls (closed arms). The other two perpendicular arms remain open (open arms) but have a 0.5cm high edge placed around the open arms to prevent the subjects from falling from the maze. The entire maze apparatus is elevated 30cm above the floor on a plus shaped steel frame stand. The video camera for recording movement is mounted to the steel frame suspended above the maze. A schematic representation of the elevated plus maze apparatus with dimensions and apparatus setup is depicted below (see Image 1. & 2).



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7. Using video tracking software, analyse the recorded behaviour of the subject

References

File, S.E., Lippa, A.S., Beer, B., Lippa, M.T., 2004. Animal tests of anxiety. Curr Protoc Neurosci Chapter 8, Unit 8 3.

ACEC Policy and Guidelines

ACEC Chair

